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(56) Documents Cited
GB 1574208 A GB 1486001 A GB 1335261 A
EP 0391554 A1 EP 0286293 A1 EP 0244088 A2
EP 0087319 A1 US 4265889 A

(58) Field of Search
UK CL (Edition L) C2C CWE
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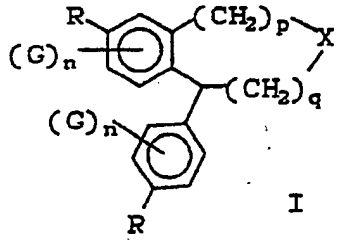
(54) HIV integrase inhibitors

(57) Compounds of the general formula:



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wherein
n is 0-3;
p is 1-2;
q is 1-2;
X is CH_2, O or N-R^1, and R^1 is H, C_{1-4} alkyl or C_{3-5} cycloalkyl;
R is (a) C_{1-6} alkyl;
(b) C_{1-6} alkoxy;
(c) hydroxyl;
(d) halogen; (e) CN;
(f) NO_2;
(g) $NHSO_2CH\ddot{I}$; or
(h) COOH;
G is H or R,
and salts or hydrates thereof have HIV integrase inhibiting activity.

18
14
36

GB 2 271 566 A

TITLE OF THE INVENTION

DIBENZYLHETEROCYCLIC OR DIBENZYLCYCLOALKYL DERIVATIVES AS INHIBITORS OF HIV INTEGRASE

BACKGROUND OF THE INVENTION

A retrovirus designated human immunodeficiency virus (HIV) is the etiological agent of the complex disease that includes progressive destruction of the immune system (acquired immune deficiency syndrome; AIDS) and degeneration of the central and peripheral nervous system. This virus was previously known as LAV, HTLV-III, or ARV. A common feature of retrovirus replication is the insertion by virally-encoded integrase of proviral DNA into the host cell genome, a required step in HIV replication in human T-lymphoid cells. Integration is believed to occur in three stages: cleavage of two nucleotides from the 3' termini of the linear proviral DNA; covalent joining of the recessed 3' OH termini of the proviral DNA at a staggered cut made at the host target site; repair synthesis by host enzymes.

Nucleotide sequencing of HIV shows the presence of a pol gene in one open reading frame [Ratner, L. et al., Nature, 313, 277(1985)]. Amino acid sequence homology provides evidence that the pol sequence encodes reverse transcriptase, an integrase and an HIV protease [Toh, H. et al., EMBO J. 4, 1267 (1985); Power, M.D. et al., Science, 231, 1567 (1986); Pearl, L.H. et al., Nature 329, 351 (1987)].

It is known that some antiviral compounds act as inhibitors of HIV and are effective agents in the treatment of AIDS and similar diseases, e.g., azidothymidine or AZT. Applicants demonstrate that the compounds of this invention are inhibitors of HIV integrase, probably by inhibiting its endonucleolytic cleavage activity rather than its binding function. The particular advantage of the present invention is highly specific inhibition of HIV integrase. The compounds of the present do not inhibit a variety of other protein-nucleic acid interactions, including enzymatic reactions involving HIV reverse transcriptase, mammalian topoisomerase I, mammalian topoisomenase II, Eco RI endonuclease, or mammalian polymerase II, as well as other related interactions, e.g., involving HIV TAT protein.

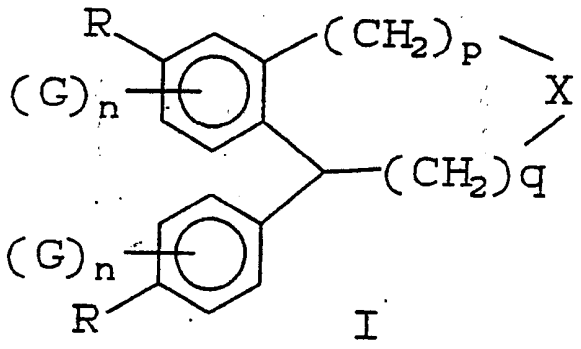
BRIEF DESCRIPTION OF THE INVENTION

Compounds of formula I, as herein defined, are disclosed. These compounds are useful in the inhibition of HIV integrase, the prevention of infection by HIV, the treatment of infection by HIV and in the treatment of AIDS and/or ARC, either as compounds, pharmaceutically acceptable salts or hydrates (when

appropriate), pharmaceutical composition ingredients, whether or not in combination with other antivirals, anti-infectives, immunomodulators, antibiotics or vaccines. Methods of treating AIDS, methods of preventing infection by HIV, and methods of treating infection by HIV are also disclosed.

DETAILED DESCRIPTION OF THE INVENTION AND PREFERRED EMBODIMENTS

This invention is concerned with compounds of formula I, combinations thereof, or pharmaceutically acceptable salts thereof, in the inhibition of HIV integrase, the prevention or treatment of infection by HIV and in the treatment of the resulting acquired immune deficiency syndrome (AIDS). Compounds of formula I are defined as follows:



I

wherein

n is 0-3;

p is 1-2;

q is 1-2;

X is CH_2, O or N-R^1, and R^1 is H, C_{1-4}alkyl or C_{3-5}cycloakyl;

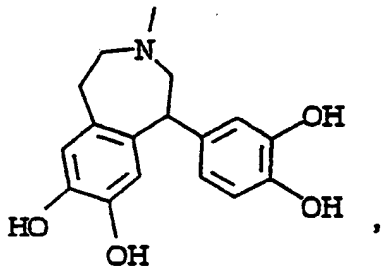
R is (a) C_{1-6} alkyl;
(b) C_{1-6} alkoxy;
(c) hydroxyl;
(d) halogen;
(e) CN;
(f) NO_2;
(g) $NHSO_2CH_3$; or
(h) COOH;

G is H or R,

or pharmaceutically acceptable salt or hydrate thereof.

A preferred compound of the present invention is Compound A as follows:

A:



,

L-619,323,

7,8-Dihydroxy-1-(3,4-dihydroxyphenyl)-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepine,

or pharmaceutically acceptable salt or hydrate thereof.

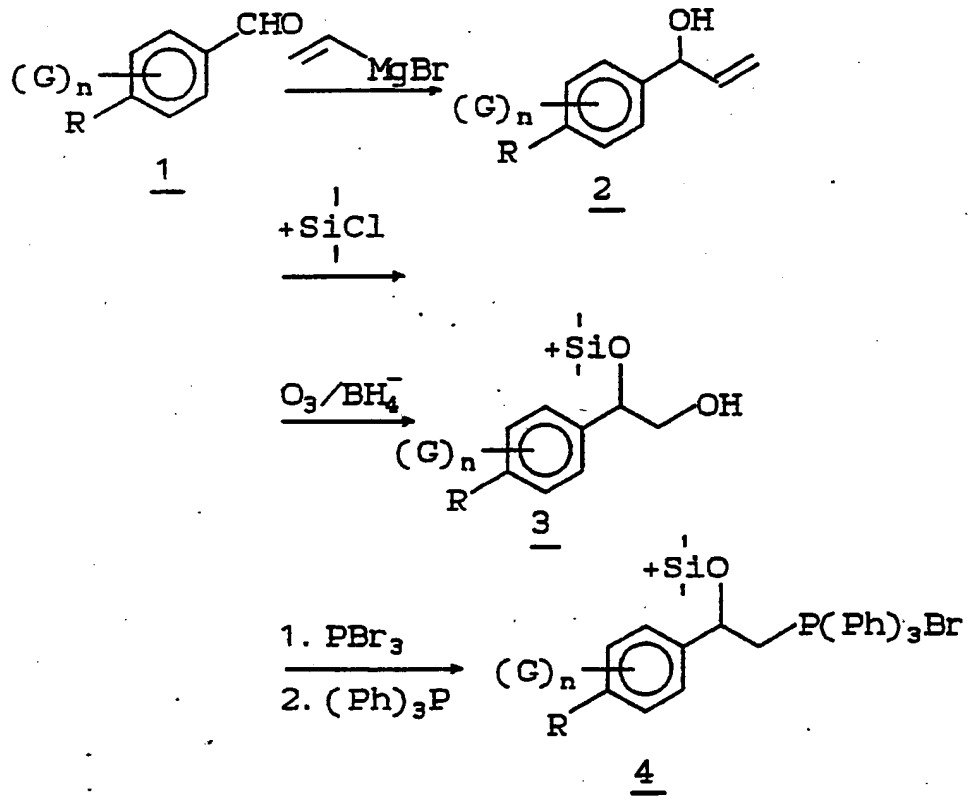
The compounds of the present invention may have asymmetric centers and may occur, except when specifically noted, as racemates, racemic mixtures or as individual diastereomers, or enantiomers, with all isomeric forms being included in the present invention.

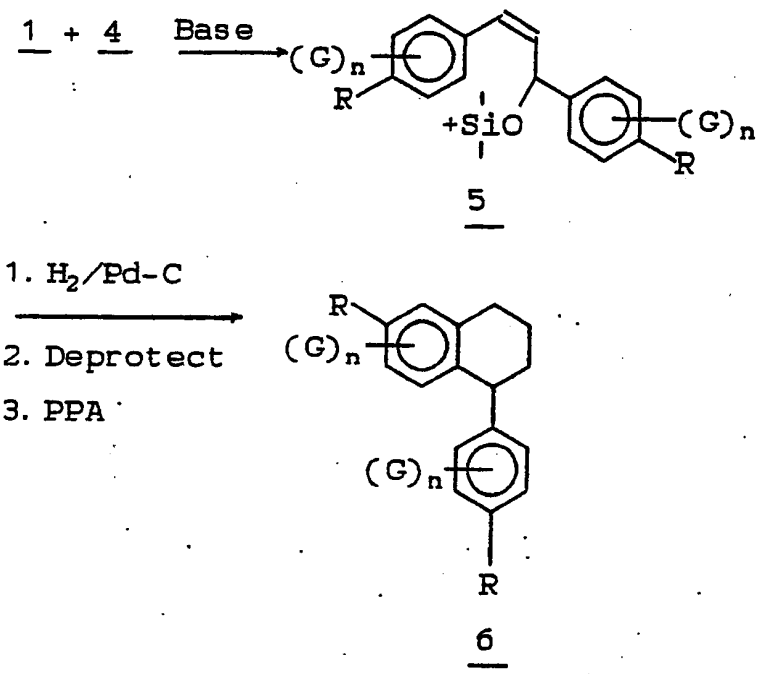
When any variable (e.g., G, R, etc.) occurs more than one time in any constituent or in formula I, its definition on each occurrence is independent of its definition at every other occurrence. Also, combinations of substituents and/or variables are permissible only if such combinations result in stable compounds.

As used herein except where noted, "alkyl" is intended to include both branched- and straight-chain saturated aliphatic hydrocarbon groups having the specified number of carbon atoms.
"Halogen" or "halo" as used herein, means fluoro, chloro, bromo and iodo.

The compounds of the present invention can be synthesized by the following methods.

SCHEME I



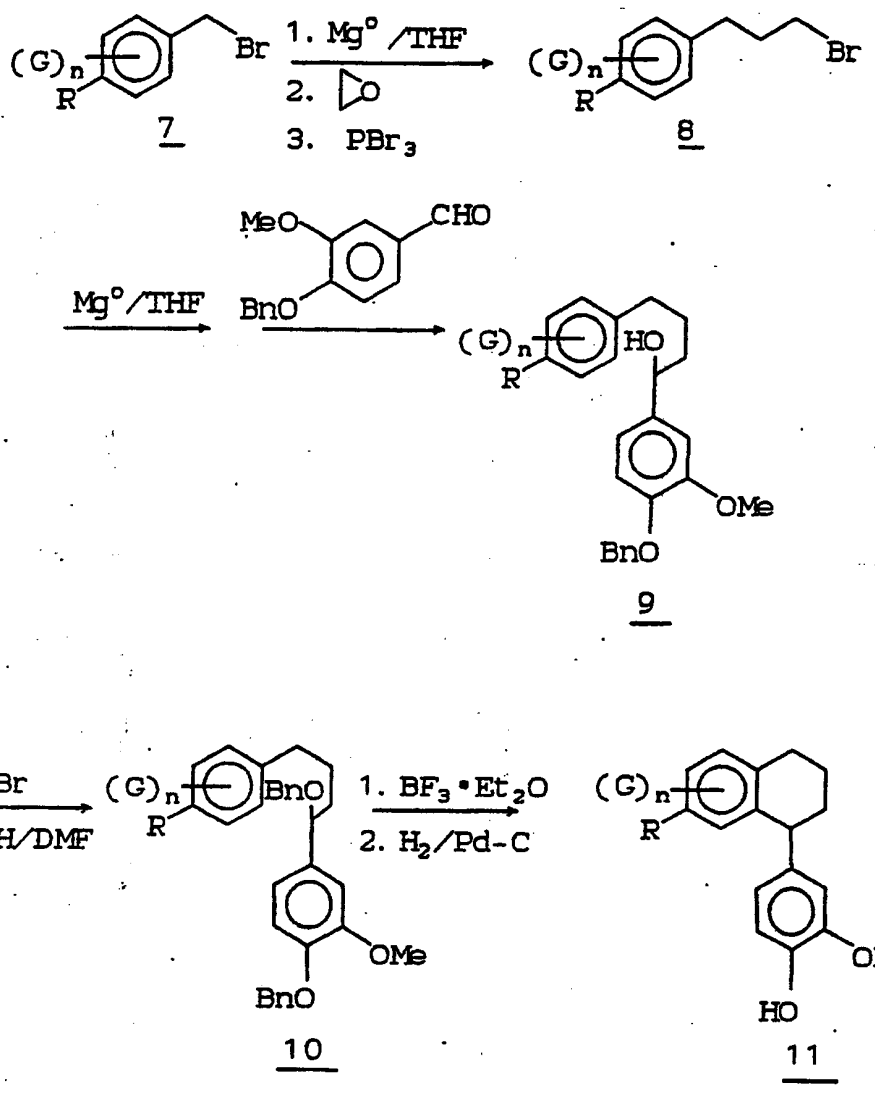


One method of synthesizing the compounds of the present invention is found in Scheme I. The phenyl aldehyde reactant is treated with an alkylene Grignard reagent to give 2. Protection of the hydroxyl, followed by ozonolysis in the presence of a reducing agent affords 3. The Wittig approach can then be used, e.g. the aldehyde 1 is reacted with 4, which is a

benzyl substituted with alkylene triphenyl phosphorane. The resulting condensation product 5 yields an unsaturated alkylene bridge, which is then hydrogenated, deprotected, then subjected to acid-catalyzed cyclodehydration, to yield 6. Further deprotection of R or G groups may or may not be needed, as the case may be. For example, OH groups protected with methyl substituents may be dealkylated by reaction with pyridine hydrochloride. It will be understood that the appropriate protecting groups will be added to prevent undesired side reactions with G or R substituents.

When $X=NR^1$ cyclodehydration is conducted with phosphorus oxychloride in the Bischler-Napieralski reaction instead of polyphosphoric acid (PPA). See Examples 1-6, as well as Grethe, b. (ed.) Isoquinolines Wiley New York 1981, pp. 142-161.

SCHEME II


(G)n
R
Br
7
1. Mg° /THF
2.
3. PBr3
8
MeO
CHO
BnO
Mg°/THF
HO
OMe
9
BnBr
NaH/DMF
BnO
1. BF3•Et2O
2. H2/Pd-C
10
11

Scheme II provides a method for synthesizing compounds of Formula I when $X=CH_2$. In Scheme II, formation of a Grignard reagent is performed by reaction of 7 with magnesium turnings. After reaction with oxirane, conversion of the resulting alcohol into the corresponding bromide 8 occurs by reaction with phosphorus tribromide. Preparation of a second Grignard reagent followed by reaction with the appropriate aromatic aldehyde, e.g. parabenzyl-vanillinaldehyde, gives the alcohol intermediate 9. Formation of a good leaving group for cyclization is accomplished by, for example, benzylation with NaH as base, to afford 10. Intramolecular Friedel-Crafts cyclization in the presence of $BF_3 \cdot Et_2O$ followed by hydrogenation provides compounds of the present invention 11. For extensive discussion of synthetic routes related to Scheme II, see, for example, Boissin, P. et al., Tetrahedron 48, 687 (1992).

The compounds of the present inventions are useful in the inhibition of HIV integrase the prevention of treatment of infection by human immunodeficiency virus (HIV) and the treatment of consequent pathological conditions such as AIDS. Treating AIDS or preventing or treating infection by HIV is defined as including, but not limited to, treating a wide range of states of HIV infection: AIDS, ARC (AIDS related complex), both symptomatic and asymptomatic, and actual or potential exposure to HIV. For example, the compounds of this invention are useful in treating infection by HIV after suspected past exposure to HIV by e.g., blood transfusion, exchange of body fluids, bites, accidental needle stick, or exposure to patient blood during surgery.

For these purposes, the compounds of the present invention may be administered orally, parenterally (including subcutaneous injections, intravenous, intramuscular, intrasternal injection or infusion techniques), by inhalation spray, or rectally, in dosage unit formulations containing conventional non-toxic pharmaceutically-acceptable carriers, adjuvants and vehicles.

Thus, in accordance with the present invention there is further provided a method of treating and a pharmaceutical composition for treating HIV infection and AIDS. The treatment involves administering to a patient in need of such treatment a pharmaceutical composition comprising a pharmaceutical carrier and a therapeutically-effective amount of a compound of the present invention.

These pharmaceutical compositions may be in the form of orally-administrable suspensions or tablets; nasal sprays; sterile injectable preparations, for example, as sterile injectable aqueous or oleagenous suspensions or suppositories.

When administered orally as a suspension, these compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and may contain microcrystalline cellulose for imparting bulk, alginic acid or sodium alginate as a suspending agent, methylcellulose as a viscosity enhancer, and sweeteners/flavoring agents known in the art. As immediate release tablets, these compositions may contain microcrystalline cellulose, dicalcium phosphate, starch, magnesium stearate and lactose and/or other excipients, binders, extenders, disintegrants, diluents and lubricants known in the art.

When administered by nasal aerosol or inhalation, these compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and may be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other solubilizing or dispersing agents known in the art.

The injectable solutions or suspensions may be formulated according to known art, using suitable non-toxic, parenterally-acceptable diluents or solvents, such as mannitol, 1,3-butanediol, water, Ringer's solution or isotonic sodium chloride solution, or suitable dispersing or wetting and suspending agents, such as sterile, bland, fixed oils, including synthetic mono- or diglycerides, and fatty acids, including oleic acid.

When rectally administered in the form of suppositories, these compositions may be prepared by mixing the drug with a suitable non-irritating excipient, such as cocoa butter, synthetic glyceride esters or polyethylene glycols, which are solid at ordinary temperatures, but liquidify and/or dissolve in the rectal cavity to release the drug.

The compounds of this invention can be administered orally to humans in a dosage range of 1 to 1000 mg/kg body weight in divided doses. One preferred dosage range is 0.1 to 100 mg/kg body weight orally in divided doses. Another preferred dosage range is 0.1 to 200 mg/kg body weight orally in divided doses. It will be understood, however, that the specific dose level and frequency of dosage for any particular patient may be varied and will depend upon a

variety of factors including the activity of the specific compound employed, the metabolic stability and length of action of that compound, the age, body weight, general health, sex, diet, mode and time of administration, rate of excretion, drug combination, the severity of the particular condition, and the host undergoing therapy.

The present invention is also directed to combinations of the HIV integrase inhibitor compounds with one or more agents useful in the treatment of AIDS. For example, the compounds of this invention may be effectively administered, whether at periods of pre-exposure and/or post-exposure, in combination with effective amounts of the AIDS antivirals, immunomodulators, antiinfectives, or vaccines, such as those in the following table.

TABLE

ANTIVIRALS

Drug Name	Manufacturer	Indication
AL-721	Ethigen (Los Angeles, CA)	ARC, PGL HIV positive, AIDS
Recombinant Human Interferon Beta	Triton Biosciences (Almeda, CA)	AIDS, Kaposi's sarcoma, ARC
Acemannan	Carrington Labs (Irving, TX)	ARC (See also immunomodulators)
Cytovene Ganciclovir	Syntex (Palo Alto, CA)	sight threatening CMV peripheral CMV retinitis
d4T Didehydrodeoxy-thymidine	Bristol-Myers (New York, NY)	AIDS, ARC
ddI Dideoxyinosine	Bristol-Myers (New York, NY)	AIDS, ARC
EL10	Elan Corp, PLC (Gainesville, GA)	HIV infection (See also immunomodulators)

Drug Name	Manufacturer	Indication
Trisodium Phosphonoformate	Astra Pharm. Products, Inc. (Westborough, MA)	CMV retinitis, HIV infection, other CMV infections
Dideoxycytidine; ddC	Hoffman-La Roche (Nutley, NJ)	AIDS, ARC
Novapren	Novaferon Labs, Inc. (Akron, OH) Diapren, Inc. (Roseville, MN, marketer)	HIV inhibitor
Peptide T Octapeptide Sequence	Peninsula Labs (Belmont, CA)	AIDS
Zidovudine; AZT	Burroughs Wellcome (Rsch. Triangle Park, NC)	AIDS, adv, ARC pediatric AIDS, Kaposi's sarcoma, asymptomatic HIV infection, less severe HIV disease, neurological involvement, in combination with other therapies.

Drug Name	Manufacturer	Indication
Ansamycin LM 427	Adria Laboratories (Dublin, OH) Erbamont (Stamford, CT)	ARC
Dextran Sulfate	Ueno Fine Chem. Ind. Ltd. (Osaka, Japan)	AIDS, ARC, HIV positive asymptomatic
Virazole Ribavirin	Viratek/ICN (Costa Mesa, CA)	asymptomatic HIV positive, LAS, ARC
Alpha Interferon	Burroughs Wellcome (Rsch. Triangle Park, NC)	Kaposi's sarcoma, HIV in combination w/Retrovir
Acyclovir	Burroughs Wellcome	AIDS, ARC, asymptomatic HIV positive, in combination with AZT.
Antibody which neutralizes pH labile alpha aberrant Interferon in an immunoadsorption column	Advanced Biotherapy Concepts (Rockville, MD)	AIDS, ARC

Drug Name	Manufacturer	Indication
L-697,661	Merck (Rahway, NJ)	AIDS, ARC, asymptomatic HIV positive, also in combination with AZT.
L-696,229	Merck (Rahway, NJ)	AIDS, ARC, asymptomatic HIV positive, also in combination with AZT.
L-735,524	Merck (Rahway, NJ)	Aids, ARC, asymptomatic HIV positive, also in combination with AZT.

IMMUNO-MODULATORS

Drug Name	Manufacturer	Indication
AS-101	Wyeth-Ayerst Labs. (Philadelphia, PA)	AIDS
Bropirimine	Upjohn (Kalamazoo, MI)	advanced AIDS
Acemannan	Carrington Labs, Inc. (Irving, TX)	AIDS, ARC (See also anti-virals)
CL246,738	American Cyanamid (Pearl River, NY) Lederle Labs (Wayne, NJ)	AIDS, Kaposi's sarcoma
EL10	Elan Corp, PLC (Gainesville, GA)	HIV infection (See also anti-virals)
Gamma Interferon	Genentech (S. San Francisco, CA)	ARC, in combination w/TNF (tumor necrosis factor)
Granulocyte Macrophage Colony Stimulating Factor	Genetics Institute (Cambridge, MA) Sandoz (East Hanover, NJ)	AIDS

Drug Name	Manufacturer	Indication
Granulocyte Macrophage Colony Stimulating Factor	Hoeschst-Roussel (Somerville, NJ) Immunex (Seattle, WA)	AIDS
Granulocyte Macrophage Colony Stimulating Factor	Schering-Plough (Madison, NJ)	AIDS AIDS, in combination w/AZT
HIV Core Particle Immunostimulant	Rorer (Ft. Washington, PA)	seropositive HIV
IL-2 Interleukin-2	Cetus (Emeryville, CA)	AIDS, in combination w/AZT
IL-2 Interleukin-2	Hoffman-La Roche (Nutley, NJ) Immunex	AIDS, ARC, HIV, in combination w/AZT
Immune Globulin Intravenous (human)	Cutter Biological (Berkeley, CA)	pediatric AIDS, in combination w/AZT
IMREG-1	Imreg (New Orleans, LA)	AIDS, Kaposi's sarcoma, ARC, PGL
IMREG-2	Imreg (New Orleans, LA)	AIDS, Kaposi's sarcoma, ARC, PGL

Drug Name	Manufacturer	Indication
Imuthiol Diethyl Dithio Carbamate	Merieux Institute (Miami, FL)	AIDS, ARC
Alpha-2 Interferon	Schering Plough (Madison, NJ)	Kaposi's sarcoma w/AZT: AIDS
Methionine-Enkephalin	TNI Pharmaceutical (Chicago, IL)	AIDS, ARC
MTP-PE Muramyl-Tripeptide	Ciba-Geigy Corp. (Summit, NJ)	Kaposi's sarcoma
Granulocyte Colony Stimulating Factor	Amgen (Thousand Oaks, CA)	AIDS, in combination w/AZT
rCD4 Recombinant Soluble Human CD4	Genentech (S. San Francisco, CA)	AIDS, ARC
rCD4-IgG hybrids		AIDS, ARC
Recombinant Soluble Human CD4	Biogen (Cambridge, MA)	AIDS, ARC
Interferon Alfa 2a	Hoffman-La Roche (Nutley, NJ)	Kaposi's sarcoma AIDS, ARC, in combination w/AZT

Drug Name	Manufacturer	Indication
SK&F106528 Soluble T4	Smith, Kline & French Laboratories (Philadelphia, PA)	HIV infection
Thymopentin	Immunobiology Research Institute (Annandale, NJ)	HIV infection
Tumor Necrosis Factor; TNF	Genentech (S. San Francisco, CA)	ARC, in combination w/gamma Interferon

ANTI-INFECTIVES

Drug Name	Manufacturer	Indication
Clindamycin with Primaquine	Upjohn (Kalamazoo, MI)	PCP
Fluconazole	Pfizer (New York, NY)	cryptococcal meningitis, candidiasis
Pastille Nystatin Pastille	Squibb Corp. (Princeton, NJ)	prevention of oral candidiasis
Ornidyl Eflornithine	Merrell Dow (Cincinnati, OH)	PCP
Pentamidine Isethionate (IM & IV)	LyphoMed (Rosemont, IL)	PCP treatment

Drug Name	Manufacturer	Indication
Piritrexim	Burroughs Wellcome (Rsch. Triangle Park, NC)	PCP treatment
Pentamidine isethionate for inhalation	Fisons Corporation (Bedford, MA)	PCP prophylaxis
Spiramycin	Rhone-Poulenc Pharmaceuticals (Princeton, NJ)	cryptosporidial diarrhea
Intraconazole-R51211	Janssen Pharm. (Piscataway, NJ)	histoplasmosis; cryptococcal meningitis
Trimetrexate	Warner-Lambert	PCP

OTHER

Drug Name	Manufacturer	Indication
Recombinant Human Erythropoietin	Ortho Pharm. Corp. (Raritan, NJ)	severe anemia assoc. with AZT therapy
Megestrol Acetate	Bristol-Myers (New York, NY)	treatment of anorexia assoc. w/AIDS
Total Enteral Nutrition	Norwich Eaton Pharmaceuticals (Norwich, NY)	diarrhea and malabsorption related to AIDS

It will be understood that the scope of combinations of the compounds of this invention with AIDS antivirals, immunomodulators, anti-infectives or vaccines is not limited to the list in the above Table, but includes in principle any combination with any pharmaceutical composition useful for the treatment of AIDS.

The compound L-697,661 is an inhibitor of HIV reverse transcriptase and is 3-([4,7-dichloro-1,3-benzoxazol-2-yl)methyl]amino)-5-ethyl-6-methyl-pyridin-2(1H)-one or pharmaceutically acceptable salt thereof. The compound L-696,229 is an inhibitor of HIV reverse transcriptase and is 3-[2-(1,3-benzoxazol-2-yl)ethyl]-5-ethyl-6-methyl-pyridin-2(1H)-one or pharmaceutically acceptable salt thereof. The compound L-735,524 is an inhibitor of HIV protease and is N-(2(R)-hydroxy-1(S)-indanyl)-2(R)-phenylmethyl-4-(S)-hydroxy-5-(1-(4-(3-pyridyl-methyl)-2(S)-N'-(t-butylcarboxamido)-piperazinyl))-pentaneamide, or pharmaceutically acceptable salt thereof.

EXAMPLE 1

1-(3,4-dimethoxybenzyl)-6,7-dimethoxy-3,4-dihydroisoquinoline

A stirred solution of N-(3,4-dimethoxyphenylethyl)-3,4-dimethoxyphenylacetamide (5g, prepared according to Weisbach, J.A. et al J. Med. Chem. 11, 752 (1968) and Wiegrebe, W., Archiv. Pharm. 297,362 (1964)), $POCl_3$ (2.5ml) in toluene (25 ml) was refluxed with stirring for 3 hours. The exotherm was cooled to room temperature, and diluted with petroleum ether (100 ml).

The supernatant was discarded, and the gummy residue washed with petroleum ether (2 x 50 ml). A volume of 100 ml ice water was added to the washed residue, the resulting mixture basified with conc. NH_4OH, and extracted with CH_2Cl_2 (4 x 50 ml). The combined CH_2Cl_2 extracts were washed with H_2O (50 ml), sat. NaCl solution (50 ml) and dried (Na_2SO_4). After 1 hour the Na_2SO_4 was filtered off, the ether solvent removed, affording a viscous, amber oil (4.6 g).

EXAMPLE 2

1-(3,4-dimethoxybenzoyl)-6,7-dimethoxy-3,4-dihydro-isoquinoline

The product of Example 1 (4.6 g) was dissolved in ethanol (15 ml) and bubbled with air while stirring overnight. A pale yellow precipitate formed, which was filtered. The filtrate was washed (2 ml ethanol), dried and the residue chromatographed on silica gel ($CHCl_3$:MeOH, 9:1). Yield: 0.9g, mp = 180-187°C. The filtrate was suspended in ethanol (15 ml) and O_2 bubbled with stirring overnight. About 0.65 g more of the pale yellow solid precipitated out, and was worked up as above. Total yield: 1.55 g.

EXAMPLE 3

1-(3,4-dimethoxybenzoyl)-6,7-dimethoxy-2-methyl-3,4-dihydroisoquinolinium iodide

The ketone product of Example 2 (1.55 g, 4.4 mmol) was suspended in ethanol (25 ml), and heated to near reflux. Methylating agent CH_3I (1.25 g, 0.55 ml, 8.8 mmol) was added and the mixture refluxed with

stirring for 5 hours. An additional 0.25 ml of CH_3I was added to drive the reaction to completion and refluxing continued overnight. At 20 hours, the mixture was cooled to room temperature and filtered. The filtrate was a yellow solid, 1.5 g, mp = 178-180°C. An analytical TLC on silica gel ($CHCl_3$:MeOH, 9:1) confirmed nearly quantitative yield.

EXAMPLE 4

1-(3,4-dimethoxy-alpha-hydroxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline hydrochloride

To the ketone iodide product of Example 3 (1.4 g), suspended in MeOH (25 ml, not completely soluble), was added stepwise $NaBH_4$ (1 g). The mixture had vigorous gas evolution. It was refluxed with stirring in a steam bath for 2 hours, cooled to room temperature and the solvent removed. The residue was taken up in 35 ml water, extracted with CH_2CH_2 (4 x 50 ml). The CH_2CH_2 extracts were combined, washed with 50 ml of saturated NaCl solution and dried (Na_2SO_4). After filtering off Na_2SO_4, and removing solvent, a viscous, glassy residue was obtained (0.9 g). The residue was dissolved in ethanol/HCl, stirred, scratched to induce crystallization, and placed in an ice water bath. The resulting white solid was filtered and dried, to afford the title compound. mp = 218-221°C (dec.)

EXAMPLE 5

7,8-Dimethoxy-1-(3,4-dimethoxyphenyl)-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepine HCl

A mixture of the product of Example 4 (14.3 g) in proprionic acid (190 ml) was warmed until the mixture turned yellow. The mixture was heated to reflux with vigorous stirring, then Zn dust (26.4 g) was added gradually over about 10 minutes. The reflux continued with vigorous stirring for 7 hours. A resulting white solid was filtered and washed with proprionic acid. The filtrate and washings were combined and dried, taken up in water (200 ml) and combined with a 50 ml H_2O wash of the white solid, to give a combined liquid wash. The combined liquid wash was basified with 10% NaOH, extracted with ether (4 x 250 ml). The combined ether extracts were washed with water (200 ml), sat. NaCl solution (200 ml) and dried (Na_2SO_4), to give a yellow oil (11.6 g). A white solid crystallized in EtOH/HCl, mp = 196°-198°C.

Anal Calcd. for $C_{22}H_{27}NO_4$

C, 64.03; H, 7.16; N, 3.56.

Found C, 63.77; H, 7.01; N, 3.37.

EXAMPLE 6

7,8-Dihydroxy-1-(3,4-dihydroxyphenyl)-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrobromide sesquihydrate, a hydrate of Compound A

The methoxy product of Example 5 (2 g, 5.1 mmol) was dissolved in CH_2Cl_2 (200 ml) and cooled in a dry ice-acetone bath. Boron tribromide (2.4 ml in 50 ml CH_2Cl_2) was added dropwise over 5 minutes, and the

resulting mixture stirred in the bath with gradually rising temperature to about 15°C. After 4 hours of stirring, methanol was added dropwise until gas evolution had ceased, giving a white precipitate. The precipitate was washed with CH_2Cl_2, filtered and dried under vacuum. The precipitate was purified by successive steps of dissolution and drying using ethanol, acetone, and finally water. mp = 192°-194.5° dec. Mass spectrometry showed a molecular ion at 301 without detectable boron.

Anal Calcd. for $C_{17}H_{19}NO_4$ + HBr + 1.5 H_2O
C, 49.89; H, 5.66; N, 3.42.
Found C, 50.37; H, 5.36; N, 3.19.

EXAMPLE 7

Annealing and Klenow Labeling of Integrase Substrate Oligonucleotide

1. HIV integrase substrate oligonucleotide (SEQ ID No:1) has been gel purified and is at a concentration of 0.65 OD/ml (21.45 µg/ml), or 1 µg = 46.6 µl

2. Lyophilize 1 µg of substrate oligo (SEQ. ID NO:1).

3. Resuspend in 48, µl dH_20,
add 1 µl 1M Tris-HCl pH 7.5
and 1 µl 5M NaCl.

4. Heat at 85°C for 15 min, take heating block out of heating unit and allow to slow cool on the bench top to room temperature, usually for 1.5 to 2

hours, affording annealed oligonucleotide (SEQ. ID No. 1), which forms a hairpin loop.

5. Divide into 5 µl aliquots and store at -20°C.

6. Prepare fresh 10X Klenow buffer:

500 µl	1M Tris-HCl pH 7.2
100 µl	1M $MgCl_2$
50 µl	5M NaCl
1 µl	1M DTT
349 µl	dH_2O
1000 µl	

7. Klenow reaction mix:

4 µl	dH_2O
2 µl	10X Klenow buffer
5 µl	annealed oligonucleotide
5 µl	^{32}p-dGTP
2 µl	1mM TTP
2 µl	Klenow enzyme (5 Units/µl)
20 µl	

Incubate at room temperature in a plexiglass shielded box for 2 hours.

8. Add 30 ul 20mM Tris-HCl pH 7.5, 100mM NaCl, 10mM EDTA buffer to the mix to stop the reaction.

9. Extract with 50 µl of Phenol/chloroform (1:1).

10. Extract with 50 µl of Chloroform/isoamyl alcohol (24:1).

11. The reaction mix is now ready for molecular sieve chromatography. It is stored at -20°C until chromatography can be done.

12. TCA precipitate aliquots coming off the column, then count both pellet and supernatant. Pool and save fractions having low counts in supernatant and high counts in the pellet, which contain labeled integrase substrate oligonucleotide.

HIV INTEGRASE ASSAY: TCA PRECIPITATION

1. On day of assay, pipet 5 µl of sample or 10% DMSO for controls into tubes kept on ice.

2. Prepare fresh reaction buffer on day of assay, and dispense eight (8) aliquots into tubes on ice for dispensing with multipipettor. Pipet 11 µl per tube.

Reaction buffer: 20 µl 1M Tris-HCl pH 7.8
3 µl 1M $MnCl_2$
3.5 µl 1.4 M Beta-mercaptoethanol (1:10 dilution of commercial stock solution)
5 µl 10 mg BSA/ml
518.5 µl dH_2O
550 µl, enough for 50 reactions

3. Thaw 1 tube of pooled integrase on ice.

4. Thaw labeled integrase substrate oligonucleotide, allow approx. 10000 cpm per reaction, dilute if necessary with 20mM Tris-HCl pH 7.5, 100 mM NaCl, 0.1 mM EDTA. Pipet 2 µl of substrate solution to be used in the assay into 208 µl of the dilution buffer, and sample 150 µl into one polypropylene tube, place on ice until end of assay (=input counts). Pipet eight aliquots into tubes on ice for dispensing with multipipettor.

5. Pipet 2 µl of HIV integrase enzyme [expressed in E. coli BL21(DE3) as described in LaFemina, R. et al., J. Virol. 65, 5624(1991)] into all reaction tubes except No Enzyme Control tubes. No Enzyme Control tubes receive 2 µl each HA Buffer B (50mM Tris-HCl pH 7.5, 10% glycerol, 1mM DTT, 0.1mM EDTA, and 1M NaCl). Immediately add 2 µl of substrate to all tubes.

6. Finger tap to mix gently. Place tubes into prewarmed racks in 37°C water bath. Incubate 60 min.

7. Add 5 µl of 1 mg tRNA/ml solution to each tube, followed by 185 µl of 11% cold TCA. Vortex hard. Place on ice at least 60 min.

8. Filter through polyvinylidene difluoride microporous filters to trap undesired TCA precipitate.

9. Determine average cpm of No Enzyme Controls (background). Determine average cpm of Enzyme Controls (100 % value). Substract background from

all samples, divide by Enzyme control value, multiply by 100, and substract from 100% to determine % Inhibition.

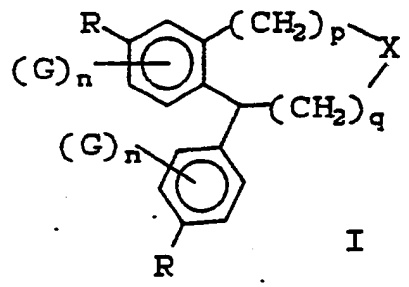
HIV Integrase Assay: Gel Cleavage

Gel cleavage was conducted according to LaFemina, R. et al., J. Virol. 65, 5624(1991), as follows. A 5'-end-labeled oligonucleotide representing the terminal 20 nucleotides of the U5 LTR plus strand (SEQ ID NO:2) was annealed with its complement (SEQ ID NO:3) and 1 ng of the resulting radiolabelled substrate was incubated with or without inhibitor for 1 h at 37°C with 80 ng (1 µl) of HIV integrase purified from E. coli BL21(DE3)/pET3c. The reaction was in 20 µl of 10 mM Tris-HCl (pH 7.8)-5 mM 2-mercaptoethanol containing 3 mM $MnCl_2$. Products were analyzed by electrophoresis on 20% sequencing gels. The positions and sizes of the substrate, 20 nucleotides, and the primary cleavage product, 18 nucleotides, are readily determined. To assay inhibition of HIV integrase, the reaction is conducted with inhibitor having various concentrations in the range of 0.1-10µg/ml. Compound A shows detectable inhibition as low as 0.5 µg/ml.

While the foregoing specification teaches the principles of the present invention, with examples provided for the purpose of illustration, it will be understood that the practice of the invention encompasses all of the usual variations, adaptations, or modifications, as come within the scope of the following claims and its equivalents.

WHAT IS CLAIMED IS:

1. A compound of the formulas:



I

wherein

n is 0-3;

p is 1-2;

q is 1-2;

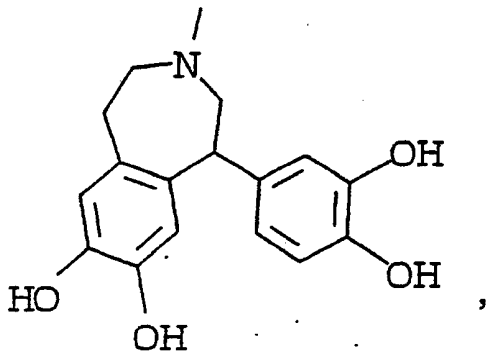
X is CH_2, O or N-R^1, and R^1 is H, C_{1-4} alkyl or C_{3-5} cycloalkyl;

R is (a) C_{1-6} alkyl;
(b) C_{1-6} alkoxy;
(c) hydroxyl;
(d) halogen;
(e) CN;
(f) NO_2;
(g) $NHSO_2CH_3$; or
(h) COOH;

G is H or R,

or pharmaceutically acceptable salt or hydrate thereof.

2. The compound



or pharmaceutically acceptable salt or hydrate thereof.

3. A method of inhibiting HIV integrase, comprising administering to a mammal an effective amount of a compound of any of Claims 1 or 2.

4. A method of preventing infection of HIV, or of treating infection by HIV or of treating AIDS or ARC, comprising administering to a mammal an effective amount of a compound of any of Claims 1 or 2.

5. A pharmaceutical composition useful for inhibiting HIV integrase, comprising an effective amount of a compound of any of Claims 1 or 2, and a pharmaceutically acceptable carrier.

6. A pharmaceutical composition useful for preventing or treating infection of HIV or for treating AIDS or ARC, comprising an effective amount of a compound of any of Claims 1 or 2, and a pharmaceutically acceptable carrier.

Patents Act 1977
Examiner's report to the Comptroller under Section 17
(The Search report)

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GB 9320785.0

Relevant Technical Fields

(i) UK Cl (Ed.L) C2C CWE

(ii) Int Cl (Ed.5) C07C, C07D

Search Examiner
P N DAVEY

Date of completion of Search
8 NOVEMBER 1993

Databases (see below)
(i) UK Patent Office collections of GB, EP, WO and US patent specifications.

(ii) ONLINE DATABASES: CAS ONLINE

Documents considered relevant following a search in respect of Claims :-
1-6

Categories of documents

X: Document indicating lack of novelty or of inventive step.

Y: Document indicating lack of inventive step if combined with one or more other documents of the same category.

A: Document indicating technological background and/or state of the art.

P: Document published on or after the declared priority date but before the filing date of the present application.

E: Patent document published on or after, but with priority date earlier than, the filing date of the present application.

&: Member of the same patent family; corresponding document.

Category	Identity of document and relevant passages		Relevant to claim(s)
X	GB 1574208	(SMITHKLINE) see eg examples 5, 8 and 11	1 at least
X	GB 1486001	(BEECHAM) see eg examples 24 and 25	1 at least
X	GB 1335261	(HOFFMANN-LA ROCHE) see examples 5, 6, 15 and 16	1 at least
X	EP 0391554 A1	(UNIV PENNSYLVANIA) see eg table 3 and examples 3, 4, 6 and 7	1 at least
X	EP 0286293 A1	(YAMANOUCHI) see eg reference. Examples 11 and 18 and examples 2, 3, 6, 7 and 11	1 at least
X	EP 0244088 A2	(SMITHKLINE BECKMAN) see eg cpd 11 and example 8	1 at least
X	EP 0087319 A1	(SMITHKLINE BECKMAN) see eg examples 1 and 2	1 at least
X	US 4265889	(SMITHKLINE) see eg example 4	1 at least

Databases:The UK Patent Office database comprises classified collections of GB, EP, WO and US patent specifications as outlined periodically in the Official Journal (Patents). The on-line databases considered for search are also listed periodically in the Official Journal (Patents).